PREVISUAL DETECTION OF STRESSED CONIFEROUS TREES

by

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I. INTRODUCTION

This is a study of the previsual detection of conifers under stress and the optimum time for detection of the different types of stress.

The first phase of the study was a visual observation of the foliage of 40-to-60-year-old Virginia pine (<u>Pinus virginiana</u> Mill.) after the tree had been killed. The trees were severed one foot above the ground and held in their position in the forest canopy by cables. Visual comparisons of killed and healthy trees were made at weekly intervals.

The laboratory phase consisted of observations of seedlings under different forms of stress: severing; girdling; drought; and fifty per cent defoliation. Severed seedlings were cut just above the soil and tied to stakes to keep them upright in their containers. A three-quarter inch band of bark was removed from the girdled seedlings. Drought was simulated by letting the containers the seedlings grew in dry out. One-half of the foliage was removed to represent fifty per cent defoliation.

Seedlings of four different coniferous seedlings were used in the laboratory studies: Virginia pine (<u>Pinus virginiana</u> Mill.); eastern white pine (<u>Pinus strobus</u> L.);

loblolly pine (Pinus taeda L.); and red spruce (Picea rubra Link.).

Observations of the stressed seedlings consisted of: needle morphology; chromatography; and photography. Needles were removed each week from each of the stressed conditions and cross sectional slides made. Two-dimensional chromatography at weekly intervals for Virginia pine and bi-weekly intervals for the other species gave pigment content information. Photographs were taken at weekly intervals of all seedlings. Four different films were used from two different camera positions. The films were: black and white; black and white infrared; color; and color infrared. Oblique photographs were taken of the seedlings from a six foot scaffolding located ten feet from them. The second position was three feet from a stressed seedling and its control.

II. LITERATURE REVIEW

Entomologists have often used the conditions of a plant's foliage to indicate the plant's susceptibility to insect attack, and as a indication that an attack is under way. Visual inspection of a plant includes observing the condition of the foliage in addition to looking for the insect.

Discoloration, wilting, blistering, skelotinization, and rolling of foliage are frequently used as signs of insect activity. All of these signs may apply to broad-leaved trees, but on conifers discoloration and some wilting are the most frequently used clues.

Due to the large acreages and limited accessibility of forest land, ground surveys are both costly and time consuming. Wert (1968) found that surveying for Douglas-fir beetle using color photographs cost less and took less time than conventional ground survey.

Fade Rates

After evaluating aerial photography for detecting southern pine beetle damage, Heller *et al.* (1959) concluded that timing of the ground survey and aerial photography was critical. The fading of tree foliage in the spring and summer could occur in a two to four week period, depending on weather, number of beetle attacks and tree vigor.

Belluschi and Johnson (1969) made a two year study of foliage fade of Douglas-fir trees attacked by the Douglas-fir beetle. Their study related variations in foliage fading between years with weather variations. A cool wet year resulted in foliage remaining green from spring to July of the following year. A warm dry year showed 70 per cent of the trees attacked in the spring turning red by October of the same year.

Doggett (1971), studying the southern pine beetle in loblolly pine, found foliage fade varying with the time of year the tree was attacked. He suggested more aerial flights in the summer than in the winter because spring and summer killed trees faded faster than winter killed trees.

<u>Pigments</u>

Gates <u>et</u> <u>al</u>. (1965) found four pigments in the leaves of most plants: chlorophyll (a); chlorophyll (b); carotene; and xyanthophyll. They also found that the lack of chlorophyll pigmentation drastically reduced the absorption of visible light by a leaf.

Techniques for Stressing Trees

Foliage fade studies have used techniques to either induce moisture stress or reduce crown foliage. Heller (1968) caged bark beetles to ponderosa pine trees to induce moisture stress. In a sutdy by Olson et al. (1969), two- and three-year-old seedlings of yellow popular and white ash were subjected to varying degrees of moisture stress by withholding water. Rhode and Olson (1970) girdled oak, red maple, and balsam popular with a chain saw to induce moisture stress. Hines (1972) cabled Virginia pine and then severed them at the base. Murtha (1968) used varying degrees of girdling and crown clipping to simulate animal damage to coniferous seedlings.

Films

Aerial surveys of large areas rely on the change in foliage conditions to detect the presence of insects. These surveys rely primarily on the change in spectral reflectance brought about by tree stress (Murtha, 1969). This change in reflectance may be collected and recorded by various camera and film combinations or by different scanning systems (Shay, 1970).

There are four major film categories being used today: black and white; infrared black and white; color; and color infrared. Each of these films are found as positive or negative image types. Positive image film records images for viewing on the film itself. The negative type produces a negative image which is generally printed as a positive.

Aerial black and white or panchromatic film has a spectral sensitivity range from 0.36u to 0.72u (Shay, 1970). Sensitivity to the red end of the spectrum is extended beyond most black and white films used on the ground. A minus-blue fliter is used with this film to reduce fogging due to blue light below 0.5u (Tarkington, 1963). Damage to foliage on black and white photographs is interpreted from pattern, texture, tone, and shape (Murtha, 1969).

Color film has a spectral sensitivity range of 0.36u to 0.7u (Sorem, 1967). On color photographs, tone is replaced by the dimension of color, which consists of three aspects: (1) hue or wave length, (2) value or brightness, and (3) chroma or saturation by a specific wavelength (Murtha, 1969). If the minimum perceivable density difference is about 0.02, not more than 200 shades of grey can be differentiated on a black and white photograph. However, under certain conditions over 7,500,000 color differences can be perceived (Laurer, 1969).

Infrared film is available in black and white or color, with sensitivity ranging from 0.36u to 0.9u. Either type of film is used with a Wratten 25 A (red) or Wratten 89 B (dark red) filter. Both of these filters cut off the blue end of the spectrum giving these films superior haze cutting capabilities (Shay, 1970).

Color infrared film records changes in plant vigor very dramatically. Healthy vegetation appears red, dying foliage appears pinkish, yellow trees appear whitish, yellow-red and red trees appear yellow (Anson, 1968).

Each of the major film types have been used to study a wide range of insect and disease outbreaks. To date none of these films has been able to previsually detect stressed foliage (Colwell, 1956; Roth, 1963; Ciesla, 1967; Heller, 1968; Houston, 1972; and Heller, 1973).

III. METHODS AND MATERIALS

This study consisted of two phases: a field study of dominant and co-dominant Virginia pine (*Pinus virginana* Mill.) and a seedling study of Virginia pine (*Pinus virginiana* Mill.), white pine (*Pinus strobus* L.), loblolly pine (*Pinus taeda* L.), and red spruce (*Picea rubra* Link.).

Field

The field study involved 40-to-60-year-old Virginia pine trees which were being used in a bark beetle study (Hines, 1972). The field study was located at the Reynolds Memorial Research Center, Patrick County, Virginia. These 700 acres are under the supervision of the Division of Forestry and Wildlife Sciences of Virginia Polytechnic Institute and State University. The Research Center is located on the southern Piedmont adjacent to the Blue Ridge Mountains. The soils are moderately sloping Hapludults (Nelson and Zillgitt, 1969). The land was first cleared for agriculture in the 1800's, abandoned about 1900, and partially logged in the early 1950's.

The Virginia pine trees were severed one foot above the ground and held upright by cables. After a sheet of plastic was placed in the kerf, another sheet of plastic and plaster of paris was used to seal the wound.

Visual comparisons of the foliage of killed trees with surrounding untreated trees of the same species were made at weekly intervals. The foliage was recorded as green, yellow, or red.

During 1971 and 1973, one tree was killed each month from April to October. During 1972, one tree was cut and killed each month from January through October.

Seedlings

The seedlings for this study were obtained from the Virginia Division of Forestry's nursery at Crimora, Virginia. The seedlings were planted in number ten cans lined with a plastic bag and filled with sterilized quartz sand. A drain hole was placed in the bottom of each can.

The seedlings were planted in March, 1973, and grown in a greenhouse until June, 1973, when they were moved out of doors. The seedlings were watered with diluted Hoagland's solution until the solution began to drain from the bottom of the cans.

In August, treatment of the seedlings was begun. The seedlings were divided into five groups: control, defoliation, drought, girdled, and severed. Each group contained ten Virginia pine, six eastern white pine, six loblolly pine, and two red spruce. The controls received no treatment. Drought was induced by discontinuing the water supply and ten days later placing a plastic bag around the can, leaving the seedling exposed. The defoliation group had 50 per cent of each seedling's foliage removed. The girdled seedlings had the outer and inner bark removed from a three-quarter inch wide strip around the stem, one inch above the root collar. In the severed treatment, seedlings were cut off and tied to stakes to keep them upright.

The tests run on the seedlings consisted of cross-sectioning of the needles to determine changes in cell structure, paper chromatography to determine pigment decay, and photography for previsual detection of the stresses imposed on the seedlings.

Cross-sectioning

During each week of the ten week study, a needle representative of the over all condition of the foliage was taken from one seedling of each species per treatment. The needles were dried in a ascending series of alcohol until 100 per cent alcohol was reached, then transferred to 100 per cent benzene, to 50 per cent benzene and 50 per cent wax, to 100 per cent wax, and finally imbeded in wax for cutting. The samples were cut into 12 micron thick sections and mounted on slides. The cross sections were stained in a two stain safranin-fast green process, described by Sass (1958).

Chromatography

Two way paper chromatography of the pigments followed a scheme modified from Sestak (Hais, 1963). Five grams of needles were taken from a seedling and ground in a blender with 50 ml. of acetone for three minutes. The acetone-pigment mixture was transferred to a 125 ml. flask and 5 ml. of petroleum ether was added. After shaking the ether and acetone-pigment mixture, the flask was filled with a saturated salt solution.

The ether-pigment mixture came to the top of the flask and was collected in a syringe. Five drops of the ether-pigment solution was placed on 20 cm. X 20 cm. Whatman #1 chromatography paper and allowed to dry.

Two developing solutions were used. The first contained ligroin-petroleum ether-acetone in a 20:5:4 ratio. The second solution contained ligroin-petroleum ether-acetone-methanol in a 40:10:4:1 ratio. The paper was developed in each solution for one hour.

The spots of the chlorophylls were cut out of the chromatogram and the pigments redissolved in two ml. of acetone. The pigment-acetone mixture was placed in a spectrophotometer and the percentage of light absorbance was measured. Chlorophyll (a) was measured at 440 millimicrons and chlorophyll (b) at 460 millimicrons.

Photography

All photography was done with a 500 EL Hasselblad with an 80 mm. f2.8 lens. The camera was equipped with four interchangable 70 mm. film magazines.

Four types of film were used: Kodak Aerochrome Infrared film 2443; Ektachrome MS Aerographic film 2448; Infrared Aerographic film 2424; Plus-X Aerographic film 2402. The aerochrome infrared 2443 and ektachrome MS aerographic 2448 films produced false color and color transparencies respectively. The infrared aerographic 2424 and the plus-x aerographic 2402 were black and white negative films.

Photographs were taken at weekly intervals between 1300 and 1500 hours. Oblique photographs were taken from a six-foot scaffolding located 10 feet from the table on which the seedlings were growing. Following the oblique photographs one seedling from each treatment was placed next to a control seedling and photographed horizontally from a distance of three feet. The same control seedling for each species was used throughout the experiment.

IV. RESULTS

Field

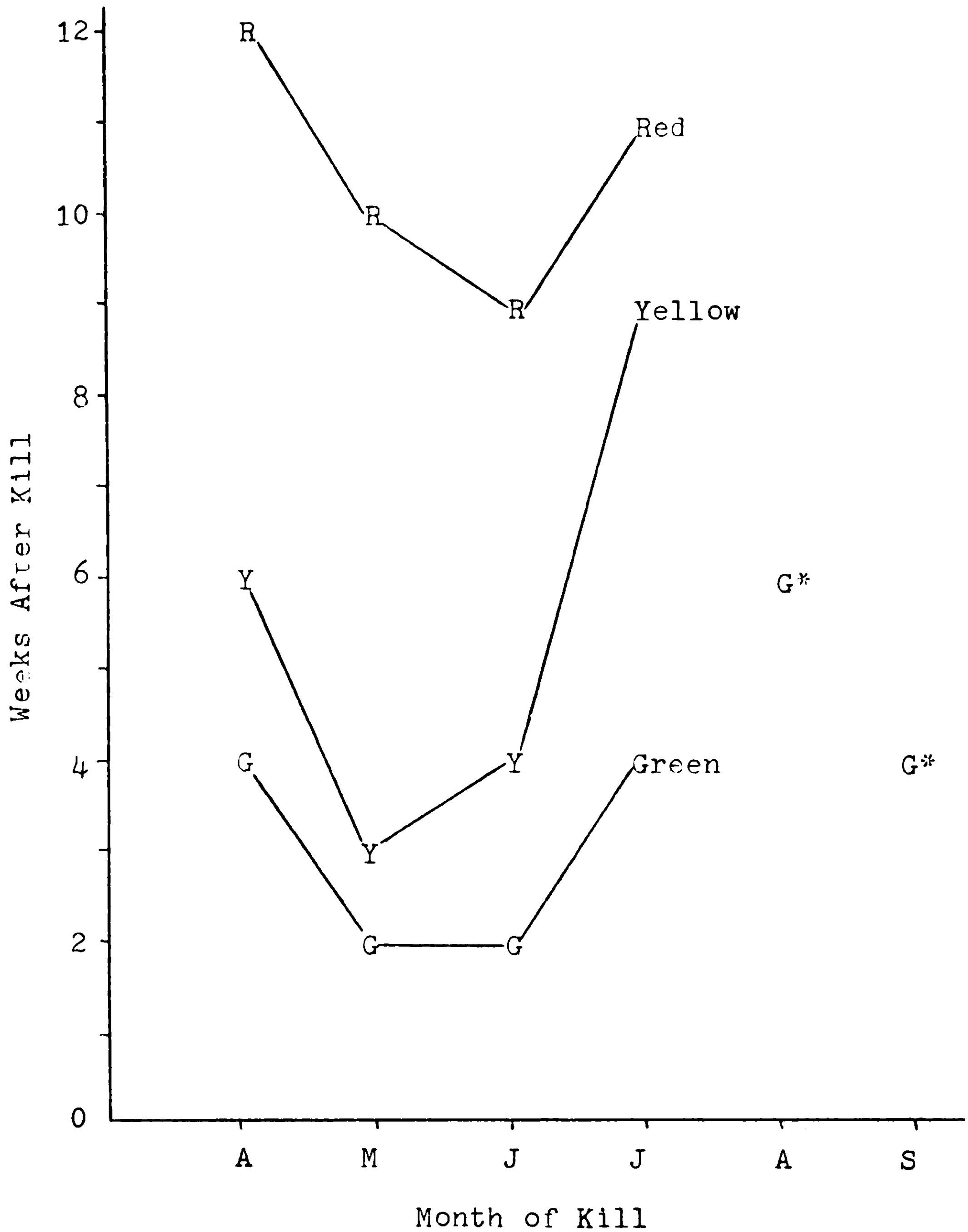
The study of the field-grown Virginia pine indicates a pattern in foliage fade rates. There is a great variation in the time the foliage appears green, yellow and red after treatment (Figures 1,2,3). Trees killed early in the year stayed in the green and yellow stages longer than those trees killed in late spring. The trees killed in May generally stayed green and yellow the shortest length of time. During June and the following months the amount of time the foliage stayed green and yellow increased.

The pattern of foliage fade plotted on a cummulative basis indicates which killed trees may be detected at what times during the year (Figure 4).

Seedlings

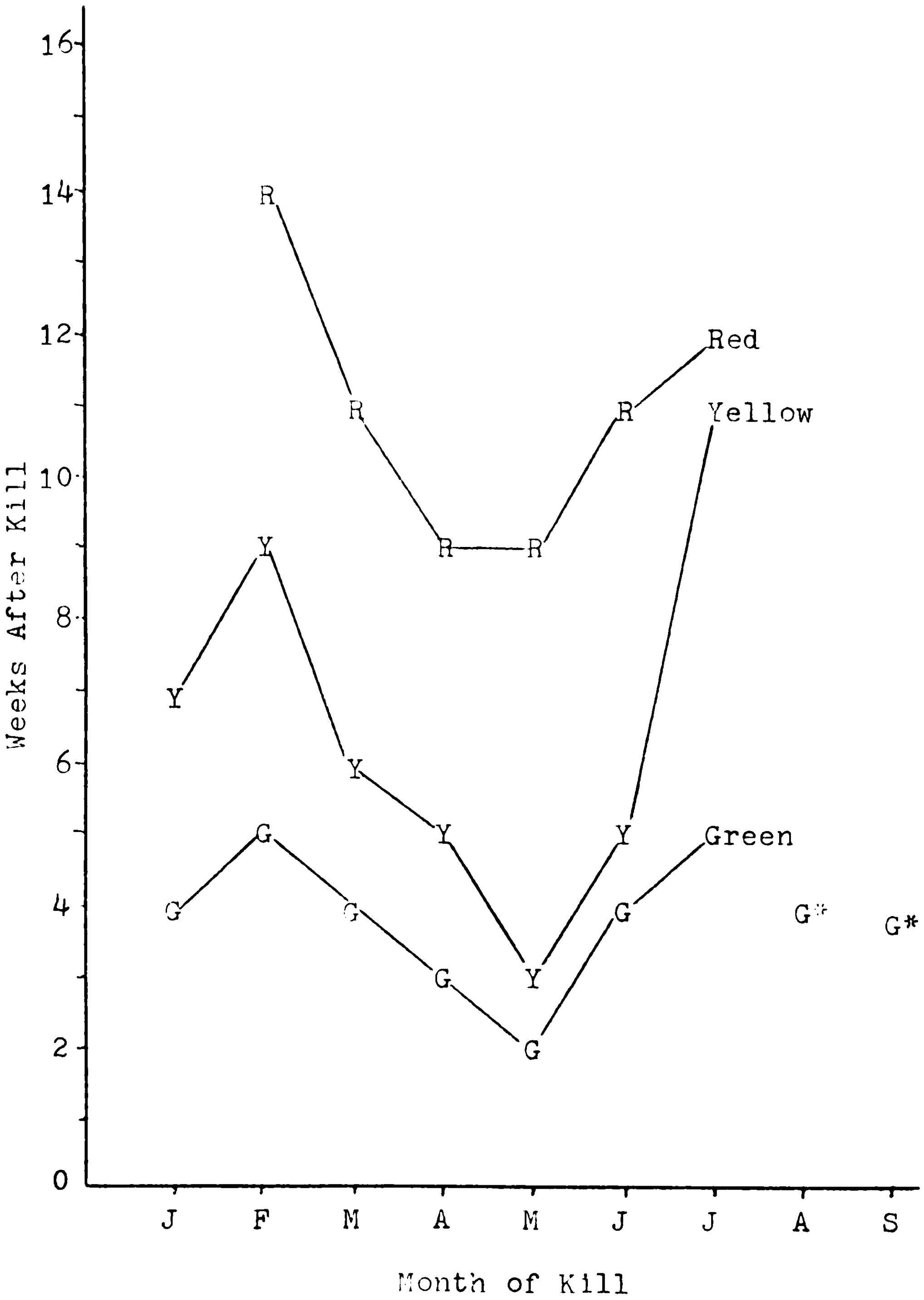
The first symptoms of foliage fade in the laboratory study were detected in the severed seedlings. Visually and photographically the foliage began to fade within two weeks after treatment. Within four weeks all needles were red on all species.

The girdled seedlings were the next to begin to fade. Visually, they were detectable at the end of four weeks. However, they did not photographically show up until the sixth week.



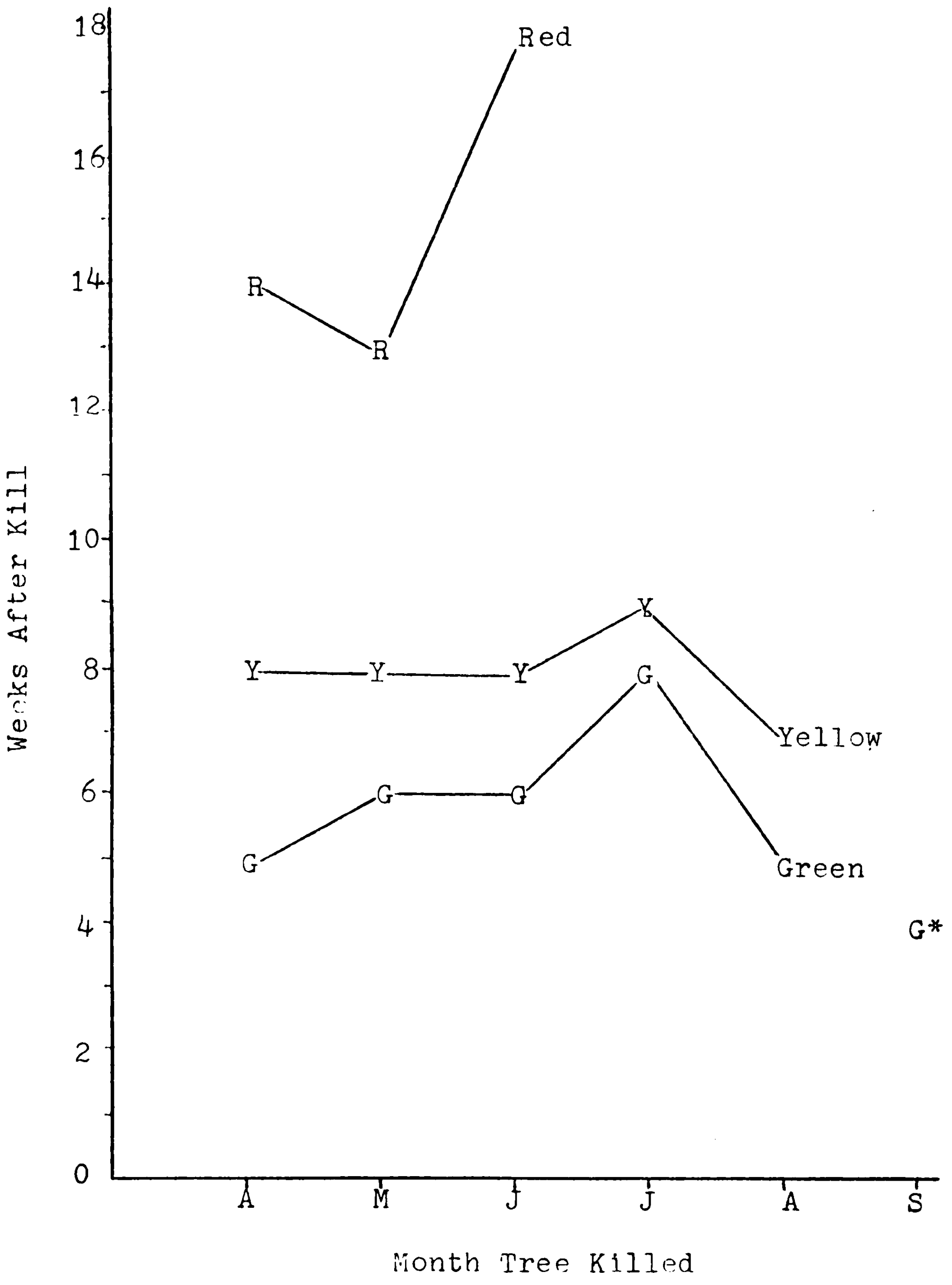
G*-Green when last observation was made.

Figure 1. Visual observations of foliage fade on treated field-grown Virginia pine during 1971.



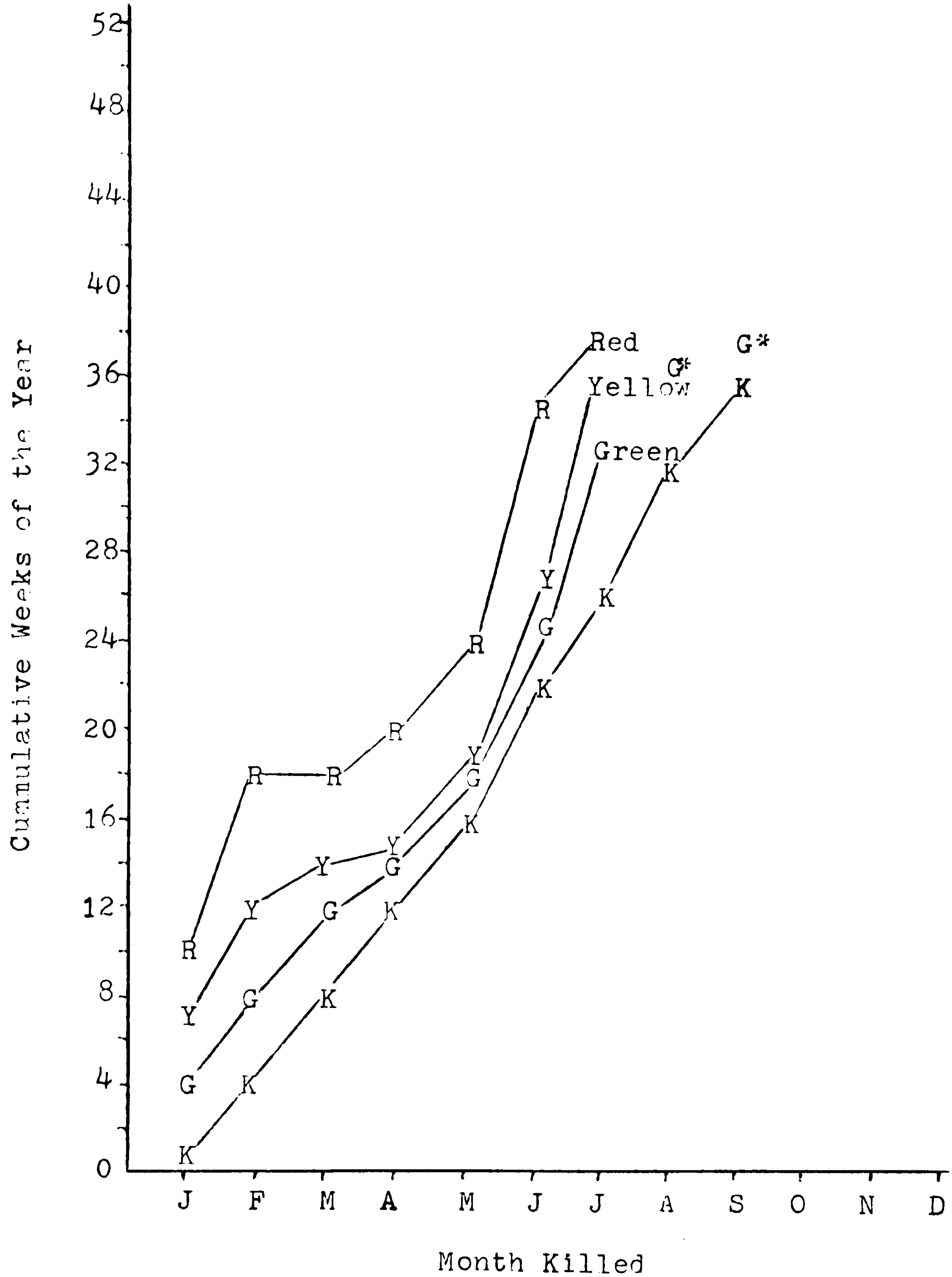
G*-Green when last observation was made.

Figure 2. Visual observations of foliage fade on treated field-grown Virginia pine during 1972.



G*-Green when last observation was made.

Figure 3. Visual observations of foliage fade on treated field-grown Virginia pine during 1973.



G*-Green when last observations were made.

Figure 4. Visual observations of foliage fade on treated field-grown Virginia pine during 1972, plotted on a cummulative basis.

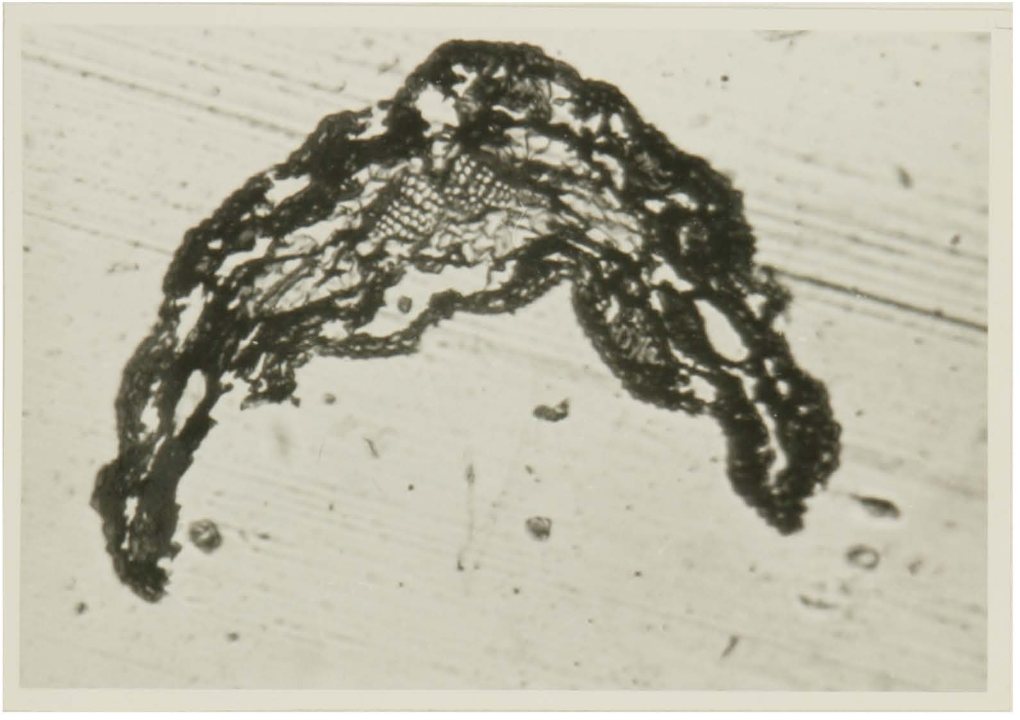
Seedlings grown under moisture stress did not begin to fade until the sixth week. However, they were not detected photographically until the eigth week. The defoliated seedlings showed no visual or photographic signs of stress during the ten weeks of this study.

Morphology

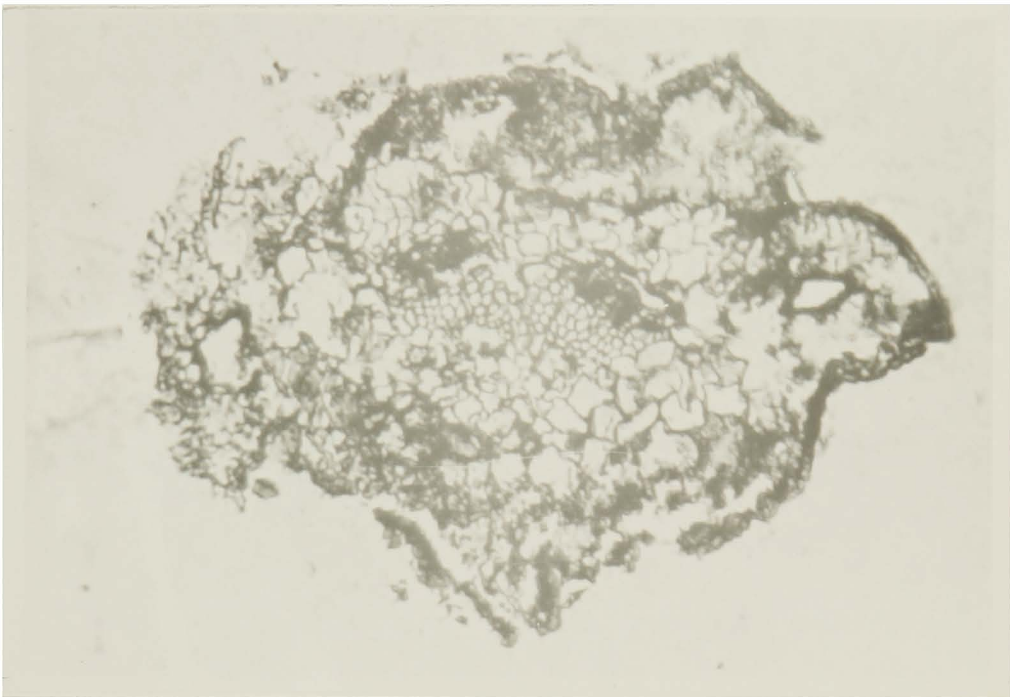
The degree of variation of internal structure within controls of the same species indicates an error in technique. The cross section of the control loblolly pine made from the needle taken at the end of the first week showed only the vascular bundle intact (Photograph 1). The cross section made from the fifth week's foliage shows part of the spongy mesophyll intact in addition to the vascular bundle (Photograph 2). The tenth week's cross-section shows the needle structure as it should appear for all of the controls (Photograph 3). These variations are too great to permit useful comparisons between and among treatments and species.

Pigments

The two-dimensional chromatography yielded four pigments. The pigments were chlorophyll (a), chlorophyll (b), a carotene, and a xyanthophyll. The chlorophylls appeared in the greatest concentration and were the easiest to detect. The chromatograms appeared the same for all species tested (Figure 5).



Photograph 1. Cross section of a needle removed from a control loblolly pine seedling one week after the study was begun.



Photograph 2. Cross section of a needle removed from a control loblolly pine seedling five weeks after the study was begun.

Photograph 3. Cross section of a needle removed from a control loblolly pine seedling ten weeks after the study was begun.

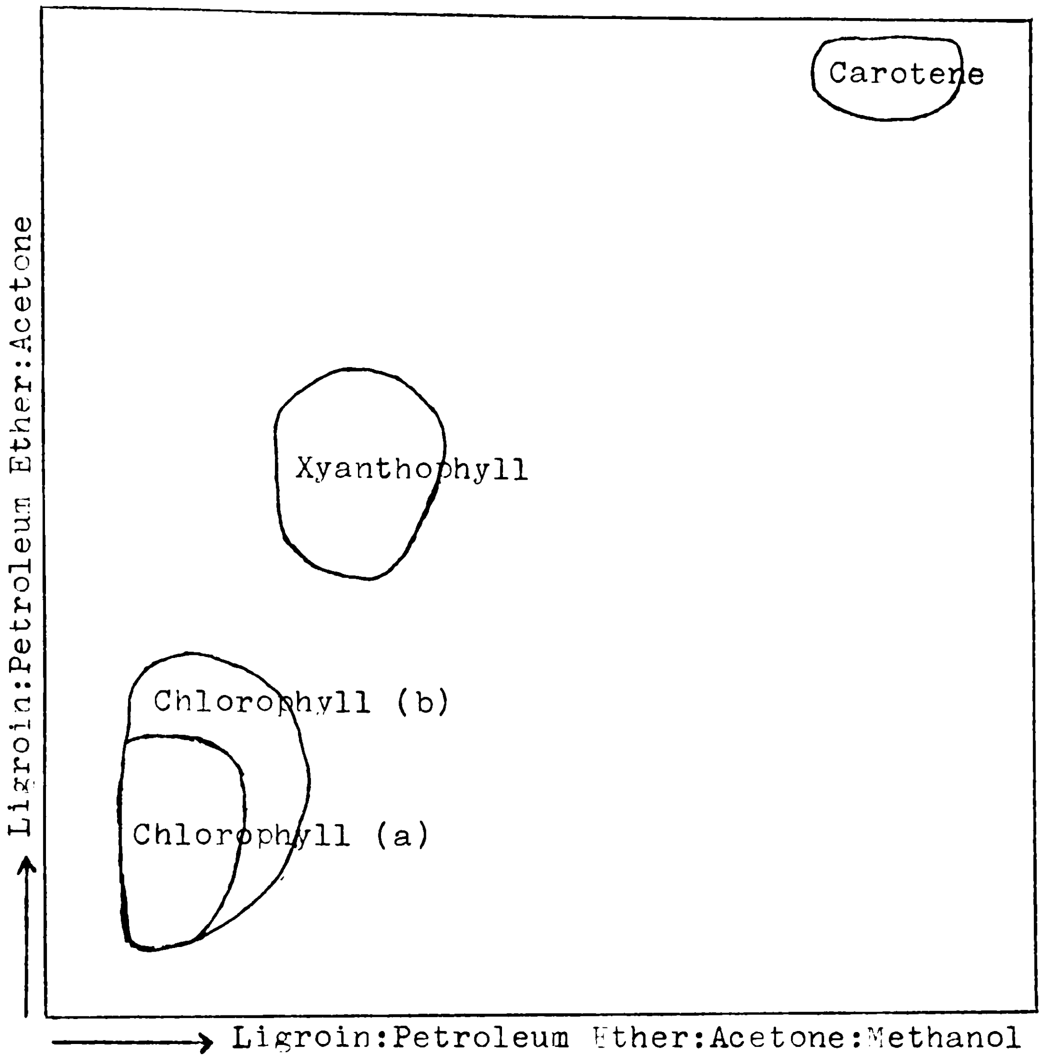


Figure 5. A schematic diagram of a chromatogram obtained from all species tested showing where the pigments were located after development.

Visual inspection of the chromatograms showed little difference between the species tested. Close visual inspections were made to determine whether any of the pigments faded faster than any of the others. However, all pigments appeared to fade at the same rate.

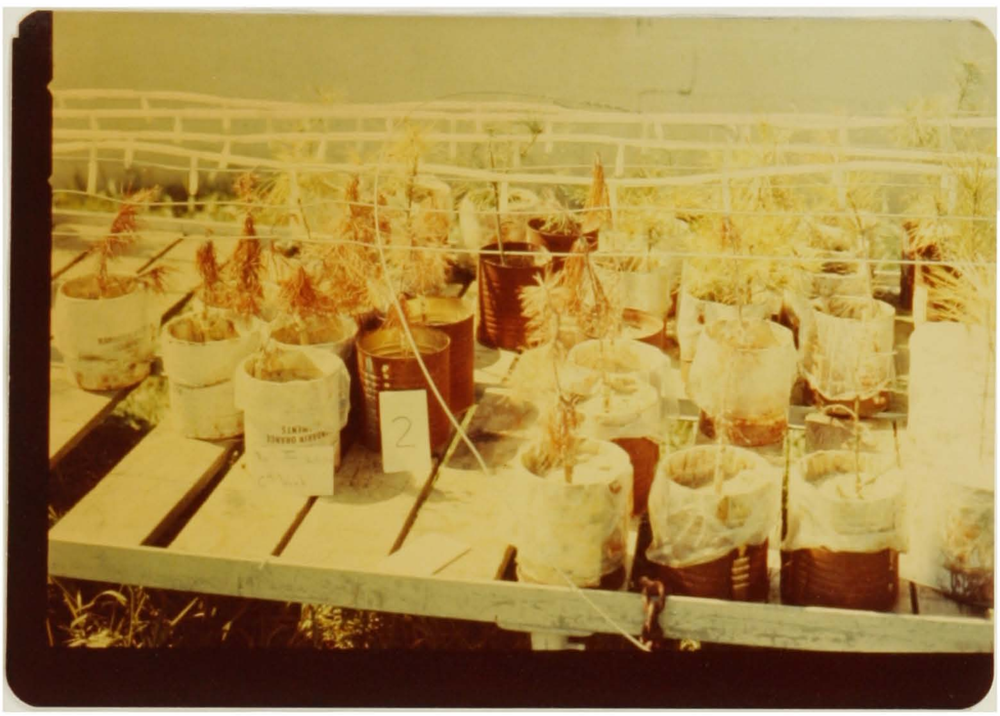
Photography

Oblique photographs of the seedlings revealed differences between healthy and stressed seedlings (Photograph 4) However, these differences did not become visible on the photographs until after the stressed symptoms were visible to the naked eye.

The prints are made from transparencies and are more yellow than the transparencies.

The oblique photograph was taken during the sixth week of the study (Photograph 4). The brown seedlings in the foreground of the photograph are the severed seedlings of all species. The seedlings in the lower right hand corner of the photograph which have the plastic covering their cans are drought treated loblolly pine. The seedlings on the left of the last row of cans at the top of the picture are the defoliated seedlings. The middle of the last row has the girdled seedlings. The seedling in the upper right hand corner of the photograph is a control loblolly pine.

Photographs taken from a distance of three feet of a stressed seedling compared with its control gave a better indication of stress than the oblique photographs. However, these photographs did not previsually detect stressed conditions (Photographs 5,6,7,8)



Photograph 4. Oblique photograph of the stressed seedlings taken the sixth week after treatment.

Photograph 5. Horizontal photograph of severed and control loblolly pine seedlings six weeks after treatment taken from a distance of three feet.

Photograph 6. Horizontal photograph of drought and control loblolly pine seedlings taken six weeks after treatment from a distance of three feet.

Photograph 7. Horizontal photograph of 50 per cent defoliated and control loblolly pine seedlings taken six weeks after treatment from a distance of three feet.

Photograph 8. Horizontal photograph of girdled and control loblolly pine seedlings taken six weeks after treatment from a distance of three feet.

V. DISCUSSION AND CONCLUSIONS

This study did not provide any means of previsually detecting dead or dying foliage. Close visual observations of the seedlings grown under stress resulted in earlier detection of dying foliage than did any of the four films tested.

The study of the field-grown Virginia pine indicates a variable pattern in foliage fade rates. Accepting the conclusion that films are at best equal to the eye in detecting foliage change, recommendations may be made for the timing of aerial surveys. The May trees generally took the shortest amount of time to go from green to yellow. This suggests a flight in late May or early June would result in detection of all trees killed that year until the first of May. A flight in late July or early August would detect dead trees up to those dying before the middle of June. With the August and September trees green into October combined with the hardwood foliage change, flights from late August to October would give little more information than the late July-early August flight.

More research is needed on the fading of field-grown trees. Work should concentrate on killing and following fade rates throughout the year. Also, data should be gathered on how fast the trees defoliate after the needles turn red. This information is needed to determine how long the tree will remain detectable as a red fader.

Four pigments were separated by the two-dimensional chromatography technique. The concentration of these pigments did not change relative to one another. With a change in the color of the foliage all pigments declined proportionately. This indicates that photographs of declining foliage will not benefit from one pigment disappearing or increasing in concentration at the expense of the other pigments.

Further work is needed to determine what happens to the pigments as the foliage turns from green to red. This investigation showed only that the pigments disappeared, not what became of the pigments.

Comparisons of the detection time between the types of stress induced show differences in the time required for stress to be detected in the foliage. This knowledge should be taken into account when surveying for insect damage. Complete disruption of the plant's functioning such as severing will give you the shortest detection time. Girdling of the tree by such insects as bark beetles may require from four to six weeks for detection. An attack on the root system and/or drought may require six to eight weeks. Removal of fifty percent of the foliage may not be detected by a color change either visually or photographically in conifers.

The results of this work are extremely preliminary and are subject to further work. Techniques of needle morphology must be improved. Chromatography of the pigments should be quantified to prevent subjective judging of pigment decay. Future photography needs a uniform background and investigation of the differences that might possibly exist between reflectance from the foliage of seedlings and the foliage of field grown trees.

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APPENDIX

Table I. The foliage color of Virginia pine (*Pinus virginiana* Mill.) killed during 1971.

Month Killed	Weeks		
	Green	Yellow	Red
April	4	2	6
May	2	1	7
June	2	2	5
July	4	5	2
August	6		
September	4		

Table II. The foliage color of Virginia Pine (*Pinus virginiana* Mill.) killed during 1972.

Month Killed	Weeks		
	Green	Yellow	Red
January	4	3	3
February	5	4	5
March	4	2	5
April	3	2	4
May	2	1	6
June	4	2	6
July	6	5	2
August	6		
September	4		

Table III. The foliage color of Virginia pine (*Pinus virginiana* Mill.) killed during 1973.

Month Killed	Weeks		
	Green	Yellow	Red
April	5	3	6
May	6	2	5
June	6	2	10
July	8	1	
August	5	2	
September	4		

Table IV. The percentage of transmittance of chlorophyll (a) extracted from Virginia pine (*Pinus virginiana* Mill.) measured at 740 u.

Treatment	Weeks After Treatment								
	1	2	3	4	5	6	7	8	9
Control	5	7	3	5	5	5	5	11	24
Girdled	3	7	11	6	5	30	17	0	0
Severed	2	1	0	0	0	0	0	0	0
Defoliated	4	9	7	4	0	11	12	0	2
Drought	5	5	7	5	3	24	9	10	6

Table V. The percentage of transmittance of chlorophyll (a) extracted from white pine (*Pinus* *strobus* L.) measured at 740 u.

Treatment	Weeks After Treatment								
	1	2	3	4	5	6	7	8	9
Control	15		6		40		30		22
Girdled	7		9		42		10		10
Severed	0		0		0		0		0
Defoliated	12		40		27		9		14
Drought	18		20		15		6		7

Table VI. The percentabe of transmittance of chlorophyll (a) extracted from loblolly pine (*Pinus taeda* L.) measured at 740 u.

Treatment	Weeks After Treatment								
	1	2	3	4	5	6	7	8	9
Control	3		4		1		7		10
Girdled	5		2		1		3		0
Severed	3		5		0		0		0
Defoliated	4		4		2		2		7
Drought	4		26		0		2		7

Table VII. The percentage of transmittance of chlorophyll (a) extracted from red spruce (*Picea rubra* Link.) measured at 740 u.

Treatment	Weeks After Treatment								
	1	2	3	4	5	6	7	8	9
Control	11		11		21				
Girdled	5		6		0				
Severed	1		0		0				
Defoliated	8		10		25				
Drought	7		10		10				

Table VIII. The percentage of transmittance of chlorophyll (b) extracted from Virginia pine (*Pinus virginiana* Mill.) measured at 760 u.

Treatment	Weeks After Treatment								
	1	2	3	4	5	6	7	8	9
Control	35	20	11	15	17	6	3	12	14
Girdled	7	19	37	12	41	32	18	0	0
Severed	2	2	2	0	0	0	0	0	0
Defoliated	9	26	25	10	11	37	8	19	5
Drought	13	11	17	13	27	19	14	20	6

Table IX. The percentage of transmittance of chlorophyll (b) extracted from eastern white pine (*Pinus strobus* L.) measured at 760 u.

Treatment	Weeks After Treatment								
	1	2	3	4	5	6	7	8	9
Control	38		27		32		55		33
Girdled	27		40		51		12		31
Severed	2		0		0		0		0
Defoliated	60		43		34		19		28
Drought	41		14		20		10		7

Table X. The percentage of transmittance of chlorophyll (b) extracted from loblolly pine (*Pinus taeda* L.) measured at 760 u.

Treatment	Weeks After Treatment								
	1	2	3	4	5	6	7	8	9
Control	7		10		15		6		9
Girdled	34		5		12		4		0
Severed	7		3		0		0		0
Defoliated	12		18		8		3		14
Drought	23		11		6		2		10

Table XI. The percentage of transmittance of chlorophyll (b) extracted from red spruce (*Picea rubra* Link.) measured at 760 u.

Treatment	Weeks After Treatment								
	1	2	3	4	5	6	7	8	9
Control	34		32		36				
Girdled	27		13		0				
Severed	1		0		0				
Defoliated	36		37		37				
Drought	26		45		17				

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PREVISUAL DETECTION OF STRESSED CONIFEROUS TREES

by

Larry Allen Alger

(ABSTRACT)

The study consisted of two parts: following the fade rates of killed field-grown Virginia pine (Pinus virginiana Mill.); and to study the effects of different stresses on seedlings.

Foliage fade rates of killed field-grown Virginia pine varied with the time of the year the tree was killed. Little variation in fade rates was found between years.

Foliage of seedlings grown under stress due to: girdling; severing; defoliating; and drought, were studied by paper chromatography, cross sectioning, and photography. The four pigments found appeared to decline proportionately as the foliage went from green to red. An error in technique resulted in uninterpretable needle cross sections. Photography using black and white, black and white infrared, color, and color infrared films gave no previsual detection of stress.